

FIG. I

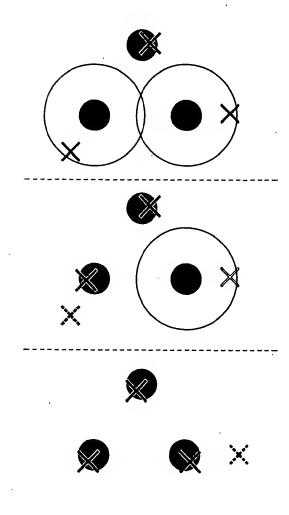
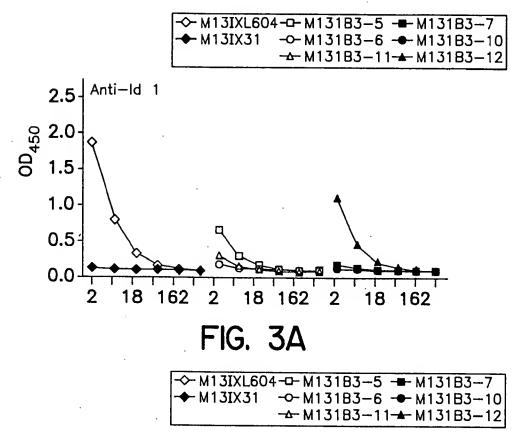
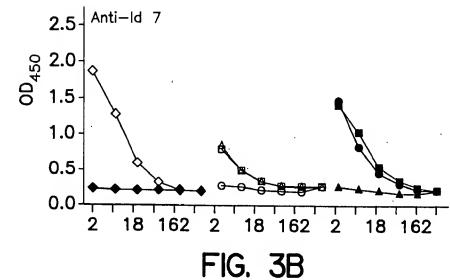
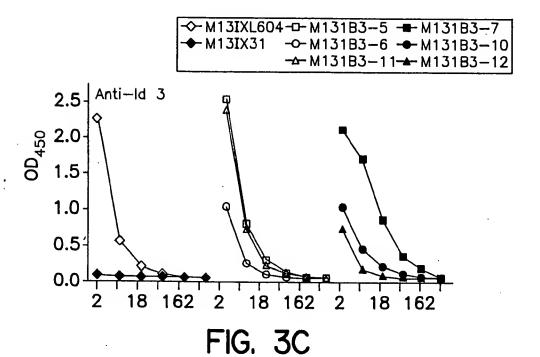
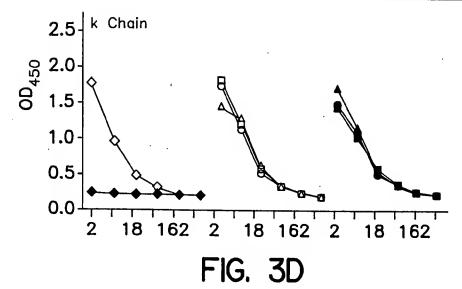


FIG. 2









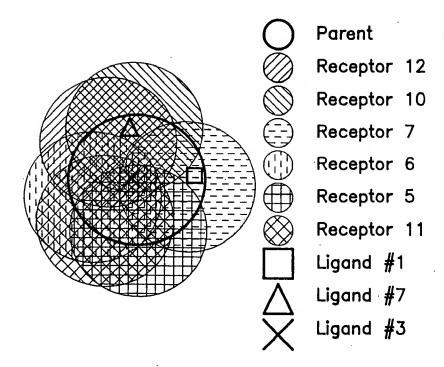
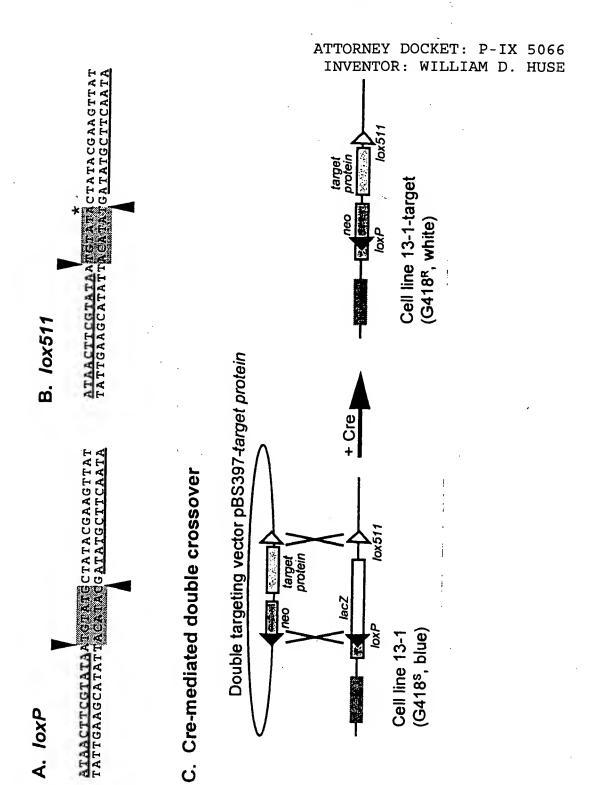


FIG. 4



DGTLLRLIQNELLAGIS ROC DEDNNLISFFQQIKS - -SEVVSTNFRDASGPAMTEIGE PWG-REFALR DPAGNCVHFVAEEQD-KPL-----GILHPNTSLKD WWDERDFAVID DDNNLISFFQQIKS-KSV-GIQETSSGYPRIHAPEL OEWGGTMAALVD DGTLLRIQNELLA 80 120 VRIHLWEASDEGWRSRSNDSPVCTGAESFIAGTASCRIEVEGIDELM LMLEFFAHPGLDP-------LASWFSCCLRLDDLAMFN 50 -VVPDNTLAWVWVRGLD回 GETLVHHEDGFAVLMCNE GEGIVFRDAGWMILQRGD SRDFVEDDFAGVVRDD DVAGAVEEWTDRES DIKKSIGHYCDKEG DFDSTAARY-ERIG 20 VTLFISAVODO--- MRMLOSI PA - - MTDQATPN MAKLTSAV * * * Sh ble Sa ble Sh ble Sa ble Tn5 ble Sa ble In5 ble Sh ble Tn5 ble

Cre recombinase-mediated targeted integration of BRP variants in 13-1 mammalian cells

400 μg/ml geneticin

Selection of site-specific integrants



Zeocin ($\mu g/ml$) \rightarrow

| 500 | 1000 | 2500 | 5000 | Comments | |
|-----|------|------|------|----------------------|--|
| + | + | + | + | Beneficial mutation | |
| + | + | + | - | Wild-type | |
| + | + | | | Detrimental mutation | |
| + | _ | _ | _ | Detrimental mutation | |
| | _ | _ | _ | Non-functional BRP | |

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| <mark>1</mark> EDDIIIATKN | | 20 | 30 | 40 | SDOCE THE |
|------------------------------|--------------------------|--------------------|------------------|--------------------------|------------|
| 60 EDDITIATKN | NGKVRGMNLT 1 | | rlgipyaqe '80 | PLGKLKENT 90 | 100 |
| WSDIWNATKY | YANSCCQNII | OQSFPGFHG: | SEMWNPNTI | DLSEDCLYL | IVWIPAP |
| 110 | 120 | 1 | 30 | 140 | 150 |
| KPKNATV <u>LIV</u> | Wiygggfqto | TSSLHVYD | GKFLARVEF | RVIVVSMNYF | Rvgalgf |
| 160 LALPGNPEAE | | | | | |
| 210 | 220 | 230 | <u>)</u> | 40 | 250 |
| SLHLLSPGSE | HSLFTRAIL(| QSGSFNAPW | AVTSLYEAI | RNRTLNLAKI | LTGCSRE |
| 260 | 270 | 280 | 290 | 3 | 00 |
| NETEIIKCL | RNKDPQEILI | Lne <u>afvvpy</u> | GTPLSVNF | GPTVDGDFL | Idmpdil |
| 310 | 320 | 330 | 340 | 350 | QEGLKIF |
| LelgQfkkt(| Qilvgvnkde | EG <u>taflvy</u> g | APGFSKDNI | NSIITRKEF(| |
| 360 | 370 | 380 | 390 | 400 | ALEFTKK |
| FPGVSEFGKI | ESILFHYTDW | VVDDQRPEN | Yrealgdv | VGDYNFICPA | |
| 410 FSEWGNNAF | 420 Fyyfehrssi | | | 450 FVFGLPLERI | RDNYTKA |
| 160 | 470 | 480 | . 490 | 500 | 510 |
| EEILSRSIV | KRWANFAKYO | GNPNETQNN | ISTSWPVFK | STEQKYLTL | NTESTRI |
| 5 | 20 | 530 | 540 | 550 | 560 |
| MTKLRAQQC | RFWTSFFPK | VLEMTGNIC | EAEWEWKA | GFHRWNNYM | MDWKNQF |
| NDYTSKKES | | | | | |